Roche 454 Sequencing Technology

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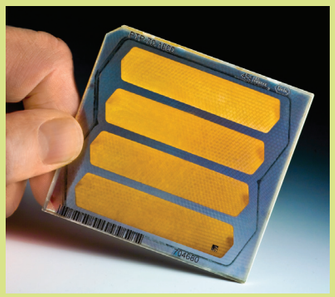
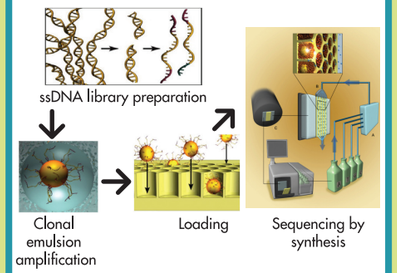
The 454 system uses emulsion PCR DNA amplification combined with pyrosequencing. The 454 instrument uses a parallel-processing approach to produce over 300 megabases (300,000,000 bases) of DNA sequence per 6-hour sequencing run. This sequencing platform differs fundamentally from Sanger sequencing in that it simplifies the upstream process to eliminate cloning, colony picking, DNA fragment labeling and other sample preparation—prior to loading on the instrument. After library construction, millions of DNA fragments are individually amplified in a single plate during emulsion PCR. Sequencing reaction chemistry occurs in the 3.6 million wells of an optical fiber slide while being imaged on the instrument during the run. The method is based on a chemical light-producing enzymatic reaction that's triggered when a molecular recognition event occurs. Sequencing of a single strand of DNA is achieved by synthesizing its complementary strand. Each time one type of nucleotide—A, C, T or G—is incorporated into the growing chain, a cascade of enzymatic reactions is initiated, resulting in a light signal detected by a CCD camera.



PicoTiter Plate

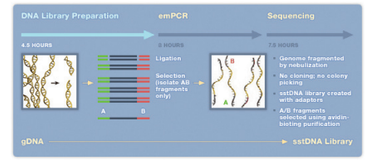
1 454 Instrument

2 454 Workflow



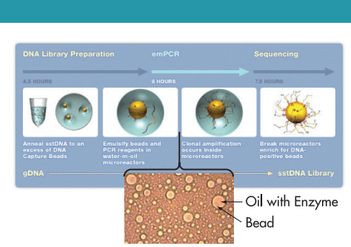
3 Library Preparation

Short adaptors (A and B) - specific for both the 3' and 5' ends - are added to each fragment. The adaptors are used for purification, amplification, and sequencing steps. Single-stranded fragments with A and B adaptors compose the sample library used for subsequent work-flow steps.



4 Emulsion PCR

The sstDNA library is immobilized onto beads. The beads containing a library fragment carry a single sstDNA molecule. The bead-bound library is emulsified with the amplification reagents in a water-in-oil mixture. Each bead is captured within its own microreactor where PCR amplification occurs. This results in bead-immobilized, clonally amplified DNA fragments.



- One DNA fragment per bead
- One bead per microreactor (water droplet in oil)
- 10 million microreactors per ml
- 10 million template copies per bead after PCR

5 PicoTiterPlate Loading

- sstDNA beads
- Apyrase
- DNA polymerase
- Enzyme beads (sulfurylase and luciferase)
- Packing beads

3,600,000 wells in 75X75 mm plate

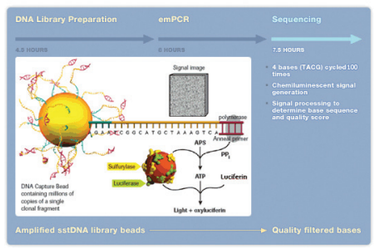
6 Pyrosequencing

Nucleotides are flowed sequentially across the plate; if a nucleotide complementary to the template strand is flowed into a well, the polymerase extends the existing DNA strand by adding nucleotide(s). Addition of one (or more) nucleotide(s) results in a reaction that generates a light signal that is recorded by the CCD camera in the Instrument. The signal strength is proportional to the number of nucleotides, for example, homopolymer stretches, incorporated in a single nucleotide flow.

Sulfurylase: diphosphate + adenylylsulfate (APS) → ATP + SO_4^{2-}(sulfate)

Luciferase: luciferin + ATP → luciferyl adenylate + PPi luciferyl adenylate + O2 ⍰ oxyluciferin + AMP + light

Apyrase: To hydrolyze excess dNTPs



7 Data Collection & Base-calling

Raw data is a series of images
Each well's intensity is extracted, quantized and normalized
Data converted into flowgrams